



## Effect of kinetic parameters on heterologous protein production: a sysbio approach

Nandy, Subir Kumar; Rattleff, Stig; Thykær, Jette; Eliasson Lantz, Anna

*Publication date:*  
2011

*Document Version*  
Publisher's PDF, also known as Version of record

[Link back to DTU Orbit](#)

*Citation (APA):*

Nandy, S. K., Rattleff, S., Thykær, J., & Eliasson Lantz, A. (2011). *Effect of kinetic parameters on heterologous protein production: a sysbio approach*. Poster session presented at 6th Danish Conference on Biotechnology and Molecular Biology, Vejle, Denmark. <http://www.biokemi.org/meetings/56>

---

### General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

# Effect of kinetic parameters on heterologous protein production: a sysbio approach

Subir Kumar Nandy<sup>1</sup>, Stig Rattleff<sup>1</sup>, Jette Thykær, Anna Eliasson Lantz\*

<sup>1</sup> Both the author have contributed equally to this work.

\* Corresponding Author: [acl@bio.dtu.dk](mailto:acl@bio.dtu.dk)

**Summary:** To improve the expression and secretion of heterologous proteins in the filamentous gram-positive bacteria, *Streptomyces lividans*, bioprocessing strategies were developed. A mathematical model was constructed to study the effects of the process kinetic parameters on the production of the model-protein RFP. The model describes the dynamics of glucose consumption and formation of biomass in addition to RFP production. The model was also extended to describe a two substrate condition.

## Background

✓ Due to its ability to secrete proteins and low protease content, *Streptomyces* have been considered as an alternative host organism for producing recombinant proteins.

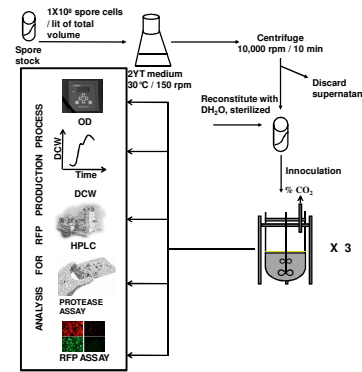
✓ In addition to genetic strategies, bioprocessing strategies are also important for improved production of protein secretion from *Streptomyces*.

✓ In this work, a mathematical kinetic model of the system for RFP production was developed. Laboratory data from batch fermentations involving growth and protein production by *S. lividans* on a single substrate, glucose was used to estimate parameters in the model. The model was then also extended to describe growth and production on double substrates, glucose and glutamate.

## Objective

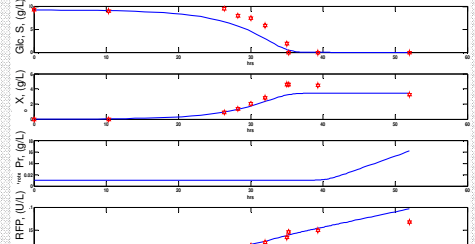
To develop a kinetic model for the prediction of protein production from *S. lividans*.

## Schematic representation of process



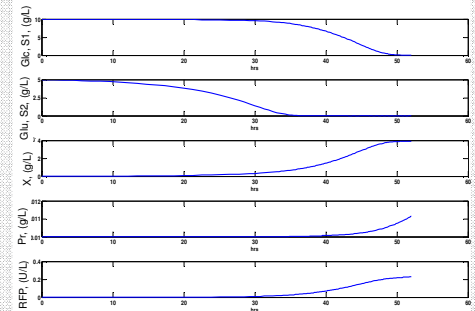
Protease assay: A2382 Sigma kit is used for this assay, Fluorescence assay: 530/25 excitation and 600/25 emission, measured from biotek-plate reader.

## Results



**Figure 1:** The single substrate kinetic model after optimization. Kinetic parameters are shown below. Lines are predictions and dots experimental data.

$\mu^m$ : 0.2, ( $h^{-1}$ ),  $K_m$ : 1.35 (g/L),  $Y_{sx}$ : 0.37. The model is validated with different initial glucose concentrations example, 20, 30 and 40 g/L.



**Figure 2:** The double substrate kinetic model as a visualization. Kinetic parameters are:  $\mu^m$ : 0.2, ( $h^{-1}$ ),  $K_m$ : 1.35 (g/L),  $Y_{sx}$ : 0.37,  $\mu^m$ : 0.1, ( $h^{-1}$ ),  $K_{m1}$ : 1.5 (g/L),  $K_{m2}$ : 1.5. The plan for near future is to validate this model with different initial substrate concentrations for example, Glc/Glu 20/10, 30/15 and 40/20 g/L.

## The Model

### ✓ Growth Rate

$$\mu = \mu^m \cdot \frac{S}{K_m + S} \quad (1)$$

$\mu$ : specific growth rate, ( $h^{-1}$ ),

$S$ : Substrate concentration, (g/L),

$\mu^m$ : max. specific growth rate, ( $h^{-1}$ ),

$K_m$ : Monod constant (g/L).

### ✓ Temperature Effect

$$\frac{dX}{dt} = \mu \cdot X - K_d \cdot X \quad (2)$$

$K_d$ : Specific death rate, ( $h^{-1}$ )

### ✓ Nutrient consumption and Product formation

Substrate = Substrate - Growth - Product - Maint.  
accumulation Feed Formation Req'd.

$$\frac{dS}{dt} = \frac{F \cdot S_0}{V} - \frac{\mu \cdot X}{Y_{sx}} - \frac{q_p \cdot X}{Y_{sp}} - m \cdot X \quad (3)$$

$Y_{sx}$ : Biomass yield on glucose

### ✓ Product Formation

Product accumulation = Formation - Destruction

$$\frac{dG}{dt} = (\alpha_G \cdot \frac{\mu^m \cdot S}{K_m + S} + \beta_G) \cdot X - K_{dG} \cdot G \quad (4)$$

$$\frac{dG}{dt} = r_G \cdot X - K_{dG} \cdot G \quad (5)$$

$$\frac{dP}{dt} = r_P \cdot X = (\alpha_P \cdot \mu + \beta_P) \cdot \left( \frac{K_{SP}}{K_{SP} + S} \right) \cdot X \quad (6)$$

$$\frac{dP}{dt} = r_P \cdot X = (\alpha_P \cdot \frac{\mu^m}{K_m + S} + \beta_P) \cdot \left( \frac{K_{SP}}{K_{SP} + S} \right) \cdot X \quad (7)$$

Where,

$r_P$ : Specific production rate of protease,

$r_G$ : Specific production rate of GFP,

$K_{dG}$ : GFP degradation rate by protease,

$r_P = (\alpha_P \cdot \mu + \beta_P) \cdot (K_{SP} / (K_{SP} + S))$  and  $r_G = (\alpha_G \cdot \mu + \beta_G)$

## Materials and Methods

*S. lividans* TK24 was used throughout the study.

The medium used in this study is (L):  $NaH_2PO_4 \cdot H_2O$  (1.38g),  $NH_4Cl$  (2.675g),  $KCl$  (0.7455 g),  $Na_2SO_4$  (0.284 g), Citric acid (0.42 g),  $MgCl_2 \cdot 6H_2O$  (0.254 g),  $CaCl_2 \cdot 2H_2O$  (0.184 g), Trace metal solution (5ml), Antifoam (sigma 204, 0.2 ml), Vitamin (1ml), Glucose- $H_2O$  (10/20/30/40 g).

## Conclusions

Comparison with experimental data demonstrated that the model is able to accurately predict the protein production process kinetics. The model will be further applied to simulate different process conditions in order to gain a better understanding of the influence of different parameters on protein production.